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Novel Nitric Oxide Donors of Phenylsulfonylfuroxan and 3-Benzyl Coumarin Derivatives as Potent Antitumor Agents

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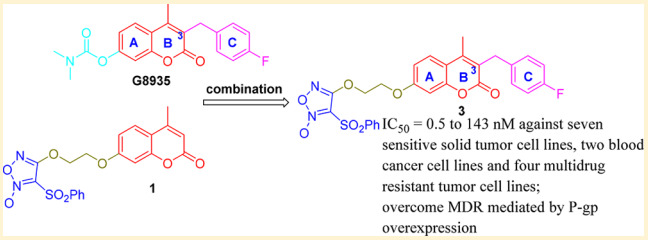
Supporting Information

ABSTRACT: In this work, five new hybrids of phenylsulfonylfuroxan merging 3-benzyl coumarin and their seco-B-ring derivatives **2**−**6** were designed and synthesized. Among them, compound **3** showed the most potent antiproliferation activities with IC_{50} values range from 0.5 to 143 nM against nine drug-sensitive and four drug-resistant cancer cell lines. Preliminary pharmacologic studies showed that these compounds displayed lower toxicities than that of lead compound **1**. Compound **3** obviously induced the early apoptosis and hardly affected the cell cycle of A2780, which was significantly different from compound **1**. Especially, it gave 559- and 294-fold selectivity antiproliferation activity in P-gp overexpressed drug-resistant cancer cell lines MCF-7/ADR and KB-V compared to their drug-sensitive ones MCF-7 and KB, implying that compounds **2**−**6** might have an extra mechanism of anti-MDR-cancer with P-gp overexpression.



KEYWORDS: *Phenylsulfonylfuroxan, 3-benzyl coumarin, anticancer, multidrug resistance, P-gp overexpression*

Nitric oxide (NO), which was reported by Furchgott and Zawadzki as an endothelium-derived relaxing factor (EDRF) in 1980,[1,2] plays important roles in diverse physiological and pathophysiological processes.[3−8] Additionally, NO can downregulate PI3K/Akt pathway and upregulate MEK/ERK pathway.[9−11] Hideo Baba et al. reported that the combined administration of NO donor and MEK inhibitor can synergistically inhibit the viability of cancer cells through downregulating both PI3K/Akt and MEK/ERK pathways.[12] (3,4-Bis(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (phenylsulfonylfuroxan) as an important NO donor was widely used in the design of anticancer agents.[13−17] We previously reported that phenylsulfonylfuroxan and coumarin hybrid **1** showed remarkable antitumor activity through multitarget mechanism containing disruption of MEK pathway. However, its MEK inhibitory activity was relatively weak.[18] Considering 4-fluorobenzyl at 3-position of coumarin skeleton in G8935, which was a MEK inhibitor,[19,20] occupied a new binding pocket in the MEK docking model,[21] we also introduced several benzyl groups covering 4-fluorobenzyl to the same position of lead compound **1** and obtained five new derivatives (**2**−**6**, Scheme 1). The structure optimization aimed at developing stronger synergistic antiproliferation activity with both NO donor and MEK inhibitory activity compared to lead compound **1**. Besides, concerning coumarin core integrity for sustaining anticancer activity, two seco-B-ring derivatives were also synthesized.

As shown in Scheme 1, compounds **8a**−**c**, synthesized according to a previously described procedure,[22,23] were treated with 2-chloro-1-ethanol to provide intermediates **9a**−**c**. Compound **9c** was reduced by stannous chloride dehydrate to form **9d** and sulfonylated with *N*-methyl-2-oxooxazolidine-3-sulfonamide[24] to obtain **9e**. Compound **11** was prepared from resorcinol via Friedel−Crafts reaction, 4-hydroxyl protection, and 2-hydroxyl methylation. After aldol condensation of **11** with benzaldehyde or 4-fluorobenzaldehyde, deprotection of the 4-hydroxyl gave **12a,b**. Seco-B-ring compounds **13a,b** were synthesized by the same procedure used to obtain **9a**−**c**. Finally, **9a**−**b**,**e** and **13a,b** were merged with phenylsulfonylfuroxan to provide compounds **2**−**6**. Meanwhile, compound **15** containing phenylsulfonylfuroxan-linker fragment was also synthesized as a reference for bioevaluation analyses. Structures of **2**−**6** were confirmed by ^{1}H NMR, ^{13}C NMR, and MS spectra. In ^{1}H NMR, the chemical shift of two alkenyl hydrogens of α,β-unsaturated ketone in compound **6** were 7.65 and 7.43 ppm, respectively, and the coupling constant is 15.8 Hz, indicating a *trans*-conformation of the ethylenic double bond structure.

Then **2**−**6** were screened for their bioactivities against nine drug-sensitive solid cancer cell lines, two hematological tumors, four drug-resistant cancer and four nontumorigenesis cell lines with lead compound **1** and NO donor **15** as references, and cisplatin, doxorubicin, gemcitabine, vincristine, SAHA, lenalidomide, and CC-885[25] were chosen as positive controls. Except for MCF-7 and KB (Table 1), compounds **2**−**6** all

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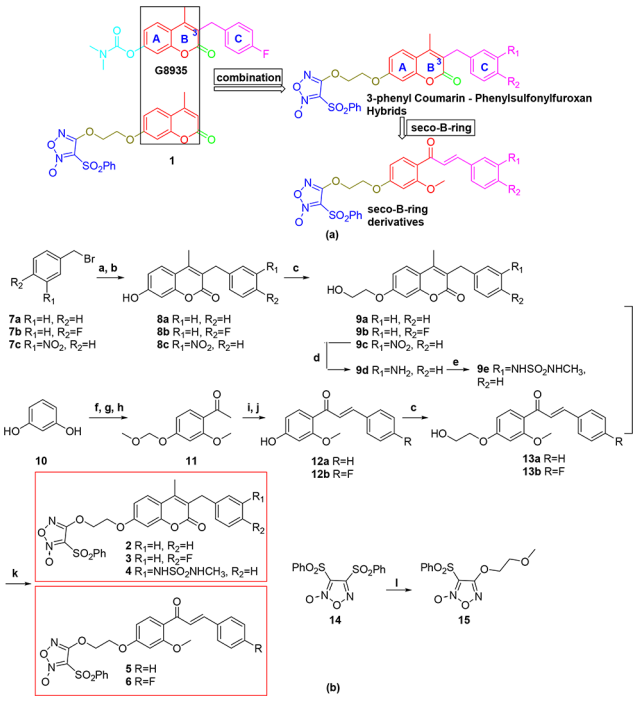
Scheme 1. Design (a) and Synthesis (b) of 3-Benzyl Coumarin and Phenylsulfonylfuroxan Hybrids and Their Seco-B-ring Derivatives[a]



[a]Reagents and conditions: (a) ethyl 3-oxobutanoate (1.0 equiv), NaH (1.2 equiv), dry THF, 60 °C, 2 h; (b) resorcinol (1.0 equiv), 70% H_2SO_4, rt, 2 h, 35−95% for two steps (a and b); (c) 2-chloro-1-ethanol (1.0 equiv), K_2CO_3 (3.0 equiv), KI (0.1 equiv), DMF, reflux, 2−10 h, 76−100%; (d) stannous chloride dehydrate (4.0 equiv), DMF, rt, 6 h, 99%; (e) *N*-methyl-2-oxooxazolidine-3-sulfonamide (2.0 equiv), NEt_3 (3.0 equiv), MeCN, 80 °C, 8 h, 99%; (f) $ZnCl_2$ (1.5 equiv), HOAc, reflux, 70 min, 51%; (g) chloromethyl methyl ether (2.0 equiv), K_2CO_3 (2.5 equiv), acetone, rt, overnight, 84%; (h) CH_3I (1.2 equiv), K_2CO_3 (3.0 equiv), DMF, 80 °C, 30 min, 82%; (i) benzaldehyde or 4-fluorobenzaldehyde (1.05 equiv), 60% KOH aqueous solution (2 mL/mM compound **11**), EtOH, 2−5 h, rt; (j) conc. HCl/EtOH = 1:25 (v/v), reflux, 30 min, 80−85% for two steps (i and j); (k) **14** (1.3 equiv), DBU (1,8-diazabicyclo[5.4.0]-undec-7-ene) (2.0 equiv), anhydrous DCM, rt, 3.5−12 h, 51−85%; (l) 2-methoxyethan-1-ol (1.1 equiv), DBU (2.0 equiv); anhydrous DCM, rt, overnight, 90%.

showed higher antiproliferation activities (0.5 to 35.8 nM) than **1** and **15**. Particularly, compound **3** with 4-fluorobenzyl at the 3-position of coumarin core was the most potent compound (0.8 to 8.3 nM). In A549, OVCA429, and MDA-MB-231, **2**−**6** all displayed significant activities in single digit nanomolar levels of IC_{50} values. While in HeLa, SKOV3, OVCA433, and A2780, **2** and **3** bearing 3-benzyl coumarin skeleton showed slightly better activities than that of their seco-B-ring derivatives (**5** and **6**). Introduction of the *N*-methylsulfomicamino group into the 3-position of phenyl group (**4**) resulted in slightly decreasing activities relative to compounds **3** and **6** bearing 4-fluorophenyl group. Additionally, they had strong antiproliferation bioactivities with a range from 5.1 to 156.8 nM of IC_{50} values against two hematological tumor cell lines MV-4-11 and MM-1S and four drug-resistant cancer cell lines A2780/CDDP, MDA-MB-231/Gem, MCF-7/ADR, and KB-V (Tables 2 and 3). Notably, the selective ratios of IC_{50} values about **3** were 559 and 294 (Table 3, 2.9 and 3.2 μM in MCF-7 and KB vs 5.1 and 11.0 nM in MCF-7/ADR and KB-V), whereas compounds **2**−**6** were almost at the same activity levels against A2780/CDDP vs A2780 and MDA-MB-231/Gem vs MDA-MB-231. The distinct selectivities presumed that new NO donors **2**−**6** probably had an extra pathway to inhibit certain MDR cancer. Moreover, **2**−**6** expressed far lower toxicities than compound **1** in HUVEC, T29, WI-38, and MCF-10A (Table 4, 0.19 to 20 μM), which indicated they have a noteworthy selectivity between tumor and nontumorigenesis cell lines.

Assay of NO release showed that **2**−**6** produced much less NO compared to control **1** (see Figure S1), and the antiproliferation activities of compound **3** declined with the increasing concentration of scavenger c-PTIO in both A2780 and A2780/CDDP (Figure 1). However, compounds **2**−**6** showed much weaker MEK inhibiting potency than that of **1**, which elucidated activities of **2**−**6** might be mainly related to the release of NO. Furthermore, compounds **3** and **6** could remarkably induce cell apoptosis but hardly affect cell cycle. Interestingly, **3** and **6** seemed to mainly induce early apoptosis, while reference **1** mainly induced late apoptosis (see Figure S3). Recently, some compounds including Dp44mT were reported to specifically inhibit the proliferation of drug-resistant cancer with P-gp overexpression.[26−31] This promoted us to detect P-gp expression of the drug-resistant and their drug-sensitive cancer cell lines mentioned above. As expected (see Figure S4),

Table 1. Antiproliferation Activities for 2−6 against Nine Solid Cancer Cell Lines[a]

	IC_{50} (nM)								
compd	HeLa	SKOV3	A549	OVCA429	OVCA433	A2780	MDA-MB-231	MCF-7	KB
15	8826	3909	4778	5173	4389	1635	1233	3503	3691
1	62.2	47.3	45.8	41.1	130.8	20.9	85.9	445	127
2	22.9	17.3	2.0	1.7	1.1	12.7	6.0	2351	4542
3	2.8	8.3	3.7	3.9	3.3	6.6	0.8	2853	3234
4	15.4	19.2	7.9	6.1	16.3	10.2	1.1	3131	1143
5	17.8	35.8	3.3	4.4	10.1	8.7	0.5	1865	2182
6	6.9	25.0	3.5	4.0	6.6	14.5	1.4	1413	1665
cisplatin	2741	1440	13220	7296	24630	2549	NA	NA	NA
doxorubicin	NA	NA	NA	NA	NA	NA	NA	879	NA
gemcitabine	NA	NA	NA	NA	NA	NA	36.4	NA	NA
vincristine	NA	NA	NA	NA	NA	NA	NA	NA	0.65

[a]MTT assay. The data are the mean of triplicate determinations. NA: not available. HeLa: human cervical cancer cell lines. SKOV3, OVCA429, OVCA433 and A2780: human ovarian cancer cell lines. A549: human nonsmall cell lung cancer cell lines. MDA-MB-231 and MCF-7: human breast cancer cell lines. KB: human oral epidermoid cancer cell lines.

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Table 2. Antiproliferation Activities for 2−6 in Hematological Tumor Cell Lines[a]

IC_{50} (nM)	1	2	3	4	5	6	SAHA	lenalidomide	CC-885
MV-4−11	28.3	24.5	27.2	66.3	29.3	29.6	NA	>20000	0.3
MM-1S	21.0	12.0	143.0	24.0	28.0	25.0	2844	NA	NA

[a]MTS assay. The data are the mean of triplicate determinations. NA: not available. MV-4−11: human myeloid leukemia cell lines. MM-1S: human myeloma cell lines.

Table 3. Antiproliferation Activities for 2−6 against Drug-Resistant Tumor Cells[a]

	IC_{50} (nM)				selectivity ratio			
compd	A2780/CDDP	MDA-MB-231/Gem	MCF-7/ADR	KB-V	$IC_{50(A2780)}/IC_{50(A2780/CDDP)}$	$IC_{50(MDA\text{-}MB\text{-}231)}/IC_{50(MDA\text{-}MB\text{-}231/Gem)}$	$IC_{50(MCF\text{-}7)}/IC_{50(MCF\text{-}7/ADR)}$	$IC_{50(KB)}/IC_{50(KB\text{-}V)}$
15	4820	4747	7845	1959	0.3	0.3	0.5	1.9
1	85.7	85.9	156.8	24.9	0.2	1.0	2.8	5.1
2	94.0	25.2	16.3	16.0	0.1	0.2	144.2	283.9
3	22.8	6.9	5.1	11.0	0.3	0.1	559.4	294.0
4	47.2	50.4	50.1	65.4	0.2	0.02	62.5	17.5
5	51.7	10.5	18.0	13.9	0.2	0.05	103.6	157.0
6	34.1	93.0	47.9	10.1	0.4	0.02	29.5	164.9
cisplatin	201370 (r.r. = 79)	NA	NA	NA	NA	NA	NA	NA
doxorubicin	NA	NA	>100000 (r.r.>113)	NA	NA	NA	NA	NA
gemcitabine	NA	>50000 (r.r. > 1373)	NA	NA	NA	NA	NA	NA
vincristine	NA	NA	NA	417.0 (r.r.=646)	NA	NA	NA	NA

[a]MTT assay. The data are the mean of triplicate determinations. r.r.: resistant ratio; r.r. = $IC_{50\ (drug\text{-}resistant\ cell\ lines)}/IC_{50\ (drug\text{-}sensitive\ cell\ lines)}$. NA: not available. A2780/CDDP: cisplatin resistant human ovarian cancer cell lines. MDA-MB-231/Gem: gemcitabine resistant human breast cancer cell lines. MCF-7/ADR: doxorubicin resistant human breast cancer cell lines. KB-V: vincristine resistant human oral epidermoid cancer cell lines.

Table 4. Antiproliferation Activities for 2−6 in Nontumorigenesis Cells

IC_{50} (μM)	1	2	3	4	5	6	cisplatin	lenalimide	CC-885
HUVEC[a]	0.13	0.44	0.27	0.19	0.41	0.36	1.10	NA	NA
T29[a]	1.8	>5	>5	>5	2.5	3.5	1.8	NA	NA
WI-38[b]	0.09	>20	>20	1.36	0.44	0.56	NA	>20	>20
MCF-10A[b]	0.66	>20	>20	NA	NA	NA	NA	>20	0.005

[a]MTT assay. [b]MTS assay. The data are the mean of triplicate determinations. NA: not available. HUVEC: human umbilical vein endothelial cell lines. T29: human immortalized but nontumorigenic ovarian epithelial cell lines. WI-38: human lung fibroblasts. MCF-10A: human nontumorigenic breast epithelial cell lines.

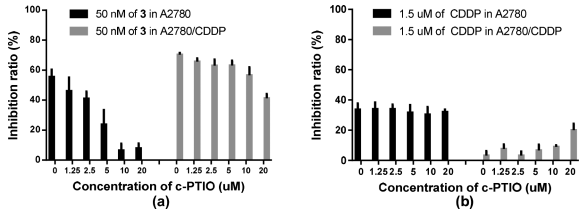

Figure 1. Antiproliferation activities of compound **3** at the concentration of 50 nM together with different concentrations of c-PTIO. Results were indicated as the mean ± SEM of two independent experiments.

P-gp expression was outstanding in KB-V and MCF-7/ADR, to which compounds **2−6** showed significant selective antiproliferation potency compared to KB and MCF-7; while P-gp overexpression was not observed in A2780/CDDP, MDA-MB-231/GEM, A2780, and MDA-MB-231, which have no relevant selective antiproliferation activities for **2−6**. Further work is under way to find the possible pharmacologic mechanism of these compounds against P-gp overexpression MDR cancer.

In conclusion, novel NO donors **2−6** not only displayed significant antiproliferation activities in nine drug-sensitive tumor cell lines but also showed strong activities against four drug-resistant tumor cell lines with nanomolar, even subnanomolar, level IC_{50} values. The less NO-releasing levels and lower toxicity on nontumorigenesis cell lines compared to lead compound **1** suggested that they had a better selectivity against malignant cells *in vitro*. Notably, some of their preliminary pharmacologic study results were different from lead compound **1**. Especially, compound **3** exhibited obvious selectivity ratios of anticancer potency in drug-resistant and their drug-sensitive cell lines MCF-7/ADR vs MCF-7 and KB-V vs KB with 559 and 294 folds, respectively. Western blot analysis discovered the overexpression of P-gp in MCF-7/ADR and KB-V, while it did not overexpress in MCF-7 and KB. The results suggested that there was a close relationship between P-gp overexpression and antiproliferation selectivity. Research of the detailed pharmacologic mechanism will further proceed for the development of desirable anticancer agents to overcome MDR mediated by P-gp overexpression.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00125.

Antiproliferative assay, inhibition activities of colony, nitrite measurement, cell apoptosis and Western blot analysis, HRMS, ^{1}H and ^{13}C NMR and HSQC spectra of compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

P-gp, P-glycoprotein; MDR, multidrug resistant; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B; MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated protein kinases; NMR, nuclear magnetic resonance; HSQC, heteronuclear single quantum correlation; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DCM, dichloromethane; SAHA, suberoylanilide hydroxamic acid; DMSO, dimethyl sulfoxide; PI, propidium iodide; HRMS, high-resolution mass spectra

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